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THE APPLICATION OF THE CONGO RED TEST TO THE DETERMINATION OF T--ETC(U)

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DEPARTMENT OF DEFENCE
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MATERIALS RESEARCH LABORATORIES
MARIBYRNONG VICTORIA

REPORT

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THE APPLICATION OF THE CONGO RED TEST
TO THE DETERMINATION OF THE TYPE AND
DEGREE OF DAMAGE TO COTTON MATERIALS

F. S. Young and W. R. Hindson

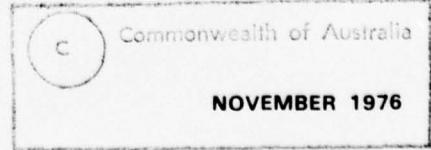


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A B S T R A C T

The Congo red microscopical test for damage to cotton developed at the Shirley Institute at Manchester has been examined and extended to meet our requirements. The procedure has been standardised and a table of observations on material damaged in the laboratory has been prepared, so that the type and degree of various forms of damage to cotton materials, in general, may be assessed on a semi-quantitative basis.

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THE APPLICATION OF THE CONGO RED TEST TO THE DETERMINATION OF
THE TYPE AND DEGREE OF DAMAGE TO COTTON MATERIALS

1. INTRODUCTION

The Congo red microscopical test for identifying damage to cotton materials was developed at the Shirley Institute at Manchester, England. It was originally reported by Bright (1) who gave a table of observations on degraded cotton. Further details were given by Clegg (2) who showed that the test depended on the spiral splitting of the cuticle on swelling in solutions of sodium hydroxide of given concentrations. With concentrations up to 9% w/w, splitting occurred after mechanical damage only, but at 11% w/w, cuticles weakened by chemical or fungal attack commenced to split and the underlying secondary cellulose could be stained during subsequent treatment with Congo red. Fibres were examined under the microscope after mounting in 18% w/w sodium hydroxide to magnify the effects. In another paper (3) Clegg discussed the examination of worn garments and gave examples of the use of the test, also extending it to flax.

We have further developed the test especially for examining materials from exposure trials to enable us to assess the cause of damage semi-quantitatively. This report describes this work and the way in which we have applied our developments to the examination of various problems.

2. EXPERIMENTAL

2.1 Materials Used

Cotton duck and cotton yarn untreated and rotproofed with copper compounds were used for the investigation of the test.

2.2 Preparation of Damaged Samples

Degraded samples of cotton duck, (mass, 400 g/m²) and cotton yarn (90 tex x 7) were prepared as follows so that the characteristic appearance on microscopical examination could be related to the loss of strength which was used as a measure of the degree of degradation.

2.2.1 Heat

Specimens were heated in an oven at 70°C for periods up to 48 days, at 110°C for 7 and 14 days, and at 150°C for 1, 4, 16, 64 and 128 hours.

2.2.2 Light

Specimens were exposed in an Atlas Weatherometer, Model HV-DL operating on a 17:3 cam, (3 min of water spray in each 20 min). Samples were withdrawn at various times up to 1152 hours.

Samples were exposed up to 12 months at Maribyrnong, Victoria, on a rack facing north inclined at 45° to the horizontal.

2.2.3 Acid

Specimens were treated by soaking in acid solutions;

Normal nitric, sulphuric and hydrochloric acids for 1-4 weeks at 15-20°C

5.8N hydrochloric acid for 1-48 h at 15-20°C

5.8N hydrochloric acid for 1-4 h at 40°C

Concentrated hydrochloric acid for 1-16 h at 15-20°C.

Only 5.8N acid at 40°C and concentrated acid produced sufficient damage for the effects to be observable in the Congo red test.

2.2.4 Hypochlorite Oxidation

Specimens were treated for 18 h at 23°C in 0.1-0.5N sodium hypochlorite buffered at pH 9 with 9 g of borax and 1 g of boric acid per litre, liquor ratio being 20:1.

2.2.5 Fungus and Mildew

Specimens were subjected to attack by strains of the following fungi:

Mucroniella echinata, *Stachybotrys atra*, *Penicillium luteum*,
P. notatum, *Aspergillus niger*, *A. ustus*, *Torula* sp.

The first three are cellulolytic organisms and specimens were withdrawn at intervals up to 14 days; the last four are non-cellulolytic and incubation was continued for 56 days.

2.3 Standardisation of Procedure

2.3.1 Preparation of Samples

Because the Congo red test is frequently used for the examination of fabrics containing various pigments and finishing agents, a preliminary treatment was given to all samples whether proofed or unproofed. The fabrics were reduced to short lengths of yarn, extracted for 3 hours with diethyl or petroleum ether, then treated for 15 min in 2% acetic acid at

40°C, for 15 min in 1% oxalic acid solution at 80°C, boiled for 1 h in 1% sodium hydroxide solution, and then boiled for 15 min in water.

This procedure has been shown by tests on proofed cotton to remove effectively most pigments and finishing agents and by tests on loomstate cotton to have no effect on observations from the Congo red test.

2.3.2 Effect of Concentration of Sodium Hydroxide used for Swelling

The concentration of sodium hydroxide determines the degree of swelling. Clegg (2) showed that with concentrations up to 7% w/w the fibre changed in shape but not in diameter. Solutions of 7-11% concentration caused rapid deconvolution and slight inward swelling without pressure on the cuticle. At concentrations of 11-18% the swelling and the pressure on the cuticle increased with increasing concentration but beyond 18% the swelling decreased. Clegg recommended the use of an 11% solution for examining fibres for chemical damage. We investigated the effect on the Congo red test of swelling damaged and undamaged cotton for 3 min at 20°C in 10%, 11%, 12% and 13% solutions and confirmed Clegg's choice of 11% as giving best differentiation between the various damaged forms with a minimum of staining of undamaged cotton.

2.3.3 Effect of Time on Swelling

Clegg recommended swelling in sodium hydroxide solution for 3 min. We examined the effect of swelling for periods of 1-5 min. The effect was slight: prolonged swelling produced a few more spirals on material damaged by heat. A swelling period of 3 min was considered best for obtaining adequate dispersion of the fibres. When the samples had been prepared as described in 2.3.1, a wetting agent in the 11% sodium hydroxide solution as used by Clegg was not required.

The amount of rinsing in water after swelling is not critical. Surplus water is removed before staining in Congo red but the sample must not be allowed to dry.

2.3.4 Effect of Temperature on Swelling

Bright and Clegg do not refer directly to the temperature of swelling but it is inferred that laboratory temperature in Britain, would have been used. The concentration of sodium hydroxide at which maximum swelling occurs becomes progressively less as the temperature decreases (5). Because of the wide range of laboratory temperatures possible in Melbourne, we investigated the effect on the Congo red test of swelling in 11% solutions at 0°, 10°, 15°, 20° and 30°C using cotton damaged by heat. Low temperatures were included because they increase the swelling considerably. The use of temperatures below 20°C resulted in the appearance of broad spirals in undamaged fibres; the frequency and intensity of these spirals increased as the temperature dropped to 0°C. Above 20°C differentiation between damaged and undamaged materials decreased. A temperature of 20 ± 1°C is appropriate for swelling tests in the laboratory.

2.3.5 Effect of Conditions of Staining

Clegg stained the swollen fibres for 10 min in 2% Congo red solution at room temperature. When the time of staining was increased up to 40 min, the amount of dye absorbed increased. Increase in temperature also increases the rate of absorption of dye and eventually fibres become uniformly stained. We found that best differentiation was obtained after staining for 10 min at 20°C.

The procedure as standardised at MRL, is :

- (i) Use a sample of about 50 mg unravelled into yarn, cleaned as described above, cut into lengths of about 6-12 mm, and thoroughly mixed.
- (ii) Immerse in 10 ml of a solution of 11% w/w (3.08N) sodium hydroxide at 20 ± 1°C for 3 min stirring gently during the first minute to assist in dispersing the fibre.
- (iii) Pour off the liquor through a 100 mesh wire gauze to collect the fibres, and rinse them with water.
- (iv) Press out surplus water with smooth filter paper.
- (v) Stain without delay by immersing for 10 min in 10 ml of a 2% aqueous solution of Congo red at 20°C ± 1°C.
- (vi) Rinse again with water collecting the fibres on a gauze.
- (vii) Press between filter papers to remove excess water.
- (viii) Mount sufficient fibres for microscopical examination, in 18% (5.39N) sodium hydroxide.

If only a few fibres are available for examination, for instance fibres taken from the edge of a hole, the test can also conveniently be done by swelling and staining them on a microscope slide.

2.4 Nomenclature Used in Descriptions

2.4.1 Degree of Staining

The staining is described as slight, moderate or intense.

2.4.2 Distribution of Staining

This may be recurring, continuous or localised.

2.4.2.1 The staining is described as *recurring* when more intense bands separated by less intense bands recur at regular intervals along the length of the fibre.

2.4.2.2 It is described as *continuous* when staining is a similar intensity throughout the length of each fibre; the staining is not necessarily all of one type.

2.4.2.3 Localised staining consists of patches of stain without regular pattern.

2.4.3 Types of Staining

2.4.3.1 Broad spirals illustrated in Fig. 1 are cuticle spirals at an angle of 70° to the fibre axis. They consist of broad spiral bands caused by occasional cleavage of the cuticle along the fibrillar structure and reverse at intervals; they are the "coarse spirals" described by Clegg (2).

2.4.3.2 Narrow spirals (Fig. 2) are cuticle spirals intermediate in width between broad and fine spirals.

2.4.3.3 Fine spirals (Fig. 3) are cuticle spirals caused by fine splitting of the cuticle as described by Clegg (2). They are observed more frequently than broad or narrow spirals.

2.4.3.4 Quick spirals (Fig. 4) are single or double spirals at an angle of about 28° with the axis of the fibre often taking the form of a figure eight. They are related to the fibrillar structure of the secondary cellulose. The staining varies from slight to fairly intense. As they occur in the interior of the fibre, they may often be detected beneath the fine spirals by focussing the microscope at a different level.

2.4.3.5 Blotchy quick spirals (Fig. 5) retain the form of the quick spiral but the staining spreads out from the quick spiral so as to form at intervals broad red patches.

2.4.3.6 Bands or patches stained uniformly red (Fig. 6) represent the final stage of damage when the cuticle is so extensively ruptured that the whole of that part of the fibre that is affected takes the stain.

2.4.3.7 Speckled staining (Fig. 7) is in the form of small patches apparently situated between the cuticle and the lumen. Under high magnification the staining appears to be below the surface of the fibres and to be associated with small cracks.

2.4.3.8 Mottled staining (Fig. 8) is patches of stain described as blotchiness by Clegg (2). It often has a mottled appearance but has no resemblance to the quick spiral.

2.4.4 Cracking and Fracture

2.4.4.1 Transverse cracks are cracks or fine lines at 90° to the axis of the fibre. They generally extend across the fibre and may cause the fibre to break up into disc-like segments. Transverse cracks may have a smooth regular outline with sharp angles at the edges as frequently observed with severe degradation by acid (Fig. 9) or they may have a more irregular outline as is observed on fibres degraded by heat (Fig. 10).

2.4.4.2 *Intensely stained broken ends* (Fig. 11) are associated with bands of intense staining where the fibres have parted the ends often being tapered. They are typical of actinic degradation.

2.4.4.3 *Cracks along the quick spiral* (Fig. 12) and *oblique splits* run diagonally (Fig. 13) across the fibre. They are associated with fungal attack and may occur as fine and short cracks in the direction of the quick spiral or open fissures that divide the fibre in this direction.

2.4.4.4 *Multiple cracking* (Fig. 14) is numerous cracks that form in various directions but obliquely rather than transversely.

2.4.4.5 *Fragmentation* (Fig. 15, 16, 17) or *disintegration* is the final stage of cracking when the fibre is breaking up into fragments.

2.4.5 *Distinctive Features of Fungal Attack*

In addition to the presence of spirals and oblique or multiple cracking there are several characteristics distinctive of fungal attack.

2.4.5.1 *Surface indentations* (Fig. 18) are small indentations on the surface of a fibre formed either at the commencement of penetration by fungal hyphae or by associated cracking.

2.4.5.2 *Hyphal penetrations* (Fig. 19) are the fine channels through which fungal hyphae enter the lumen. The whole channel can rarely be focussed at the same time so that generally only a portion in the form of a fine crack is observed. As the attack develops, the original hyphal penetrations become obscured by cracking but some surface indentations remain.

2.4.5.3 *Serrated surface* (Fig. 20) is the saw-like badly indented surface sometimes observed in microbiological deterioration when attack occurs on the surface of the fibres.

2.4.5.4 *Corrosion in the lumen* (Fig. 21) is the indentation of the secondary cellulose in the wall of the lumen as the cellulose becomes corroded by the fungi growing inside.

2.5 Assessment of Damage

Clegg described only the qualitative use of the Congo red test; an Indian report (6) indicated that the observations could be used quantitatively. In examining damaged cotton it is helpful if an estimate of the degree as well as the type of damage can be obtained especially if original material is not available. We therefore determined the losses of strength of the material degraded as described earlier and applied the Congo red test. Damage was conveniently divided into five classes :

Class 1	0-15% loss of strength
2	15-30% " " "
3	30-50% " " "
4	50-75% " " "
5	75-100% " " "

Descriptions of the results of microscopical examinations of fibres after the Congo red test are given in Table 1. This table has subsequently been used as the basis for assessing damage to cotton materials. The percentages given are the percentages of the fibres that show each particular characteristic.

When using the test, allowance must be made for dead or abnormal fibres. The cotton duck used in our experiments showed 8% of dead fibres and occasional abnormally thickened fibres or fuzz hairs. Dead fibres appear as thin-walled convoluted fibres; these fibres are neglected in assessing the percentage of fibres showing any particular effect except for the count of intensely stained broken ends when the character of the ends, stained or unstained, is quite distinctive. Occasionally abnormally thickened fibres or fuzz hairs may be present. They may show brilliant spirals even when undamaged. Allowance must be made for such fibres because the brilliance of these spirals may be confused with the effects of mildew.

Immature fibres are included in making assessments because they show the various spiral effects, the intensely stained broken ends from actinic degradation and the various characteristics of fungal attack.

Spirals are not distinctive of any particular type of damage; all the agencies studied give some spirals which are often quite brilliant on fibres affected by non-cellulolytic fungi (mildews) which grow on the waxes and the cuticle layer only. Fibres damaged by heat show brilliant spirals. This form of damage is distinguished from others by continuous staining, more fine than quick spirals at low losses of strength, and transverse cracking and segmentation with a more irregular outline at losses of strength exceeding 50%.

Actinic degradation is distinguished from the other by the recurring pattern of the staining. The effects recur at more or less regular intervals along each fibre apparently because the twist of the yarn and the structure of the fabric shield the greater part of the fibre from the full effects of the radiation. Quick and blotchy quick spirals and speckled staining occur with varying frequency but the most useful characteristic of more severe actinic degradation is intensely stained broken ends. A count of the percentage of ends that show this feature distinguishes classes 3, 4 and 5 of actinic degradation, even in the presence of damage from other sources. In addition an estimate of the percentage of fibres showing recurring staining and spirals and speckled staining, helps to give an assessment of actinic damage in the presence of damage by fungus and moderate damage by heat.

Very few distinctive features are observed on cotton degraded by acid until the loss of strength exceeds 50%. Degradation by acid is then distinguished by the smooth outline of transverse cracking, sharply broken ends and fine lines observed between transverse cracks (Fig. 9). Damage by hypochlorite oxidation is distinguished by intense staining and many spirals at losses of strength up to 50% and by fairly even staining and frequent cracking at greater losses of strength. The cracking also has a smooth regular outline similar to that observed for damage by acid.

The effects of fungal attack are readily observed, even in the presence of damage by other agencies. The amount of fungal damage is determined by the percentage of fibres that show surface indentations and related hyphal penetration and cracking, corrosion in the lumen, multiple cracking, and disintegration. The features typical of fungal attack are the penetration into the lumen through fine channels (Fig. 19), and the development of attack at that location. Some micro-organisms especially bacteria attack fibres from the outside so that the effects appear as serrated surfaces on stained fibres (Fig. 20).

Non-cellulolytic fungi (mildews) produce excessive swelling of fibres and surface roughness as well as fine and quick spirals increasing in frequency and intensity with prolonged growth of the fungi.

3. THE USE OF THE TEST AT M.R.L.

There were two main reasons for trying to refine the Congo red test at MRL. The first was to be more confident of our ability to identify the causes of degradation in cotton-based cloths under examination. Accurate diagnosis of the causes of degradation is important in order to ensure the development of the appropriate type of treatment or modification of cotton to reduce future degradation. The second reason was to try to obtain as accurate an estimate as possible both of type and degree of damage (e.g. percentage loss of strength) on goods for which no comparison material or only a few scraps or fibres were available for examination.

We have now used our modified test for the examination of many hundreds of cotton samples with, we believe, much success. Materials withdrawn from exposure trials at Maribyrnong and the sites of the Joint Tropical Research Unit at Innisfail and Cloncurry have been examined using this technique. The most common form of degradation at all three exposure sites is actinic, except that material inadequately protected against microbiological attack may be seriously degraded at the Innisfail site.

The test has enabled confirmation of the accelerating effect of many copper-based fungicides on actinic degradation of cotton. In addition, many chlorine-containing organic fungicides have been shown to be dangerous because on exposure they break down to give hydrochloric acid which then degrades the cotton. Acid degradation is not detectable at low levels in the presence of other forms of degradation but becomes apparent when loss of strength due to acid attack reaches about 50%.

Many finishing treatments for cotton that contain fungicides do permit superficial fungal growth; the Congo red test has confirmed the conclusions drawn from strength tests that such superficial growth has had no degradative effect on the cotton. Fungal and actinic degradation are readily distinguished in the presence of one another; the result from the Congo red test for these forms of degradation is quite distinct. A typical description of an examination that showed the presence of both forms of degradation on a sample of cotton duck that had been exposed at Lae, New Guinea is :

Sample	Unproofed cotton duck exposed at Lae, New Guinea for 18 months.
Loss of strength	90%
Staining	
Intensity	Moderate to fairly intense
Distribution	Recurring on about 50%
Types	
Fine spirals	A few
Quick spirals	25-75%
Blotchy quick spirals	Some
Speckled staining	5-10%
Uniform red bands	5-10%
Cracking (actinic)	
Broken ends	5%
Signs of fungal attack	
Surface indentations and hyphal penetrations	25-50%
Corrosion in lumen	About 25%
Multiple cracking	About 33%
Disintegration	Some

Damage was fairly extensive. About 5% of broken, stained ends together with recurring bands of uniform red and quick spirals indicated an assessment of Actinic, Class 3. The breaking up of fibres, together with multiple cracking, and corrosion indicated assessment of Fungus, Class 4.

The test has also been used for the examination of many cotton samples referred to us by various Government Departments and Industry. The effects of fungi, sunlight, acid, heat and overbleaching have all been demonstrated either singly or in various combinations. In general no undamaged items were available to obtain a direct measure of the degree of damage and the Congo red test has proved useful in estimating this.

4. ACKNOWLEDGEMENTS

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TABLE 1
CLASSIFICATION OF DAMAGE BY CONGO RED TEST

Type	Class 1 0-15% Loss	Class 2 15-30% Loss	Class 3 30-50% Loss	Class 4 50-75% Loss	Class 5 75-100% Loss
UNDAMAGED					
Staining	S1 on most, more intense on thin walled con- voluted fibres. Sometimes a few intense localised patches.				
Types	Occasional narrow or fine spirals. Nil				
Cracking					
HEAT					
Staining	S1-Mod	S1-Mod	Mod-F Int	Mod-Int	Int
Intensity	Continuous, a little localised	Continuous, a little localised	Continuous	Continuous	Continuous
Distribution					
Types	5-20% 0-10% A few Nil -	About 50% 0-10% A few Nil -	50-75% 50-75% 5-10% A few Some -	50-75% 50-75% 10-50% 10-50% 10-50% -	A few A few A few Most Most Frequent

TABLE 1
(Cont.)

Type	Class 1 0-15% Loss	Class 2 15-30% Loss	Class 3 30-50% Loss	Class 4 50-75% Loss	Class 5 75-100% Loss
ACTINIC					
Staining Intensity	S1-Mod	S1-Med	S1-F Int	S1-Int	Int
Distribution	S1 recur	More than 50% recur	50-75% recur	Most recur	Recur
Types					
Fine spirals	0-10%	A few	A few	A few	Very few
Quick spirals	5-10%	10-50%	About 50%	10-50%	Some
Blotchy quick spirals	A few	10-25%	10-25%	25-50%	Some
Speckled Staining	A few	A few	About 25%	A few	Neg
Uniform red bands	Nil	A few	5-25%	50-75%	Most
Cracking					
Broken stained ends	Nil	0-15%	15-20%	75-100%	

TABLE 1
(Cont.)

Type	Class 1 0-15% Loss	Class 2 15-30% Loss	Class 3 30-50% Loss	Class 4 50-75% Loss	Class 5 75-100% Loss
ACID					
Staining Intensity	S1	S1-Mod	S1-Mod	Mod	Mod-Int
Distribution	Continuous	Continuous Some intense localised	Continuous Some intense localised	Continuous or even	Continuous or even
Types					
Fine spirals	A few	A few	A few	10-50%	Very few
Quick spirals			No distinctive features of damage	25-50%	Very few
Mottled staining			until in excess of 50% loss of strength	Some	Very few
Uniform red				A few	Most
Cracking				5-10%	25-75%
Transverse cracks					
Cleanly broken fibres				A few	Many

TABLE 1
(Cont.)

Type	Class 1 0-15% Loss	Class 2 15-30% Loss	Class 3 30-50% Loss	Class 4 50-75% Loss	Class 5 75-100% Loss
HYPOCHLORITE OXIDATION					
Staining intensity	Sl-Mod	Mod-F Int	Mod-Int	Int-V Int	Very Int
Distribution	Continuous	Continuous	Continuous	Continuous	Continuous
Types					or even
Fine spirals	10-20%	75%	25%	Nil	
Quick spirals	10-20%	75%	-	Nil	
Blotchy quick spirals	A few	25-50%	Over 75%	Neg	
Uniform red	Nil	A little	25-50%	Most	100%
Cracking					
Broken fibres with cracks	Nil	0-50%	50-75%	100%	Most in fragments

TABLE 1
(Cont.)

Type	Class 1 0-15% Loss	Class 2 15-30% Loss	Class 3 30-50% Loss	Class 4 50-75% Loss	Class 5 75-100% Loss
CELLULOYTIC FUNGUS					
Staining intensity	Sl-Mod	Mod	Mod-F Int	Mod-Int	Int
Distribution	Continuous a little localised	Continuous but patchy	Continuous some localised	Continuous	Continuous
Types					
Fine spirals	A few	25-50%	25-50%	A few	A few
Quick spirals	A few	25-50%	25-50%	A few	A few
Uniform red	-	-	-	Most	Most
Surface indentations and hyphal penetrations	A few	5-25%	Over 25%	25-50%	Over 50%
Corrosion in lumen	Neg	A little	10%	10-25%	25%
Multiple cracking	Nil	A few	0-20%	20-50%	50-100%
Disintegration	Nil	Neg	A little	Some	Some

TABLE 1
(Cont.)

Type	Class 1 0-15% Loss	Class 2 15-30% Loss	Class 3 30-50% Loss	Class 4 50-75% Loss	Class 5 75-100% Loss
MILDEW	Staining intensity Distribution Types	S1-F Int Continuous a little localised	Mod-F Int Continuous		

KEY:

Loss = Loss of strength

F Int = Fairly intense

S1 = Slight

Int = Intense

Mod = Moderate

Neg = Negligible

CONGO RED TEST ON COTTON

Magnification X250



FIG. 1 - Broad spirals.



FIG. 2 - Narrow spirals.



FIG. 3 - Fine spirals.



FIG. 4 - Quick spirals.



FIG. 5 - Blotchy quick spirals.



FIG. 6 - Uniform red bands.

CONGO RED TEST ON COTTON

Magnification X250



FIG. 7 - Speckled staining.



FIG. 8 - Mottled staining.



FIG. 9 - Transverse cracks with smooth regular outline from damage by acid.

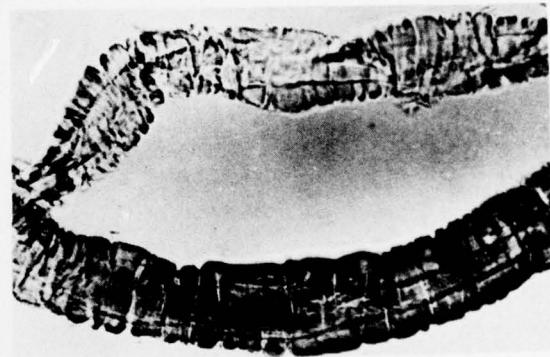


FIG. 10 - Transverse cracks from damage by heat.

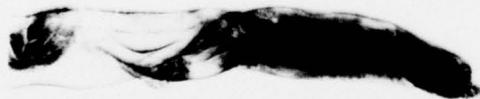


FIG. 11 - Intensely stained broken ends from actinic degradation.



FIG. 12 - Cracks along the quick spiral from fungal attack.

CONGO RED TEST ON COTTON

Magnification X250



FIG. 13 - Oblique split from fungal attack.



FIG. 14 - Multiple cracking from fungal attack.



FIG. 15 - Fibre commencing to disintegrate from fungal attacks.

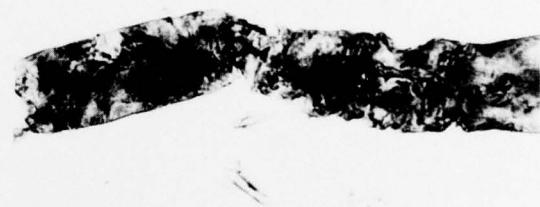


FIG. 16 - Fragmentation from fungal attack.



FIG. 17 - Fragmentation from fungal attack.



FIG. 18 - Surface indentations, commencement of fungal attack.

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FIG. 19 - Hyphal penetrations from fungal attack.



FIG. 20 - Serrated surface from microbiological attack.



FIG. 21 - Corrosion in the lumen from fungal attack.

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